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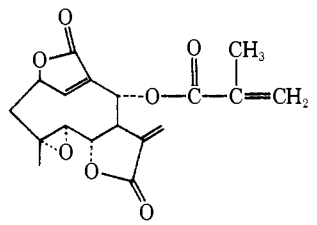
Synthesis of Alkyl-Substituted α,β-Unsaturated γ-Lactones as Potential Antitumor Agents

Gary A. Howie, Peter E. Manni, and John M. Cassady*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received February 8, 1974*

A series of alkyl-substituted di- and monolactones including (*E,E*)-3,3′-(alkanediylidene)bis[dihydro-2(3*H*)-furanones] 9–12 and the monolactones 18 and 19 was synthesized by reaction of α-(triphenylphosphoranylidene)-γ-butyrolactone with appropriate aldehydes. The reaction of this ylide with formaldehyde gave α-methylene-γ-butyrolactone (20). These compounds were tested for antitumor activity as part of a study to determine the influence of β substituents and distance between alkylating sites on the antitumor activity of α,β-unsaturated lactones. The testing was carried out in standard NCI screens and the compounds possessed ED_{50} values of 16–110 μg/ml against cells derived from human carcinoma of the nasopharynx (KB) and were inactive against L1210 lymphoid leukemia in the mouse.

Extensive screening of plant extracts has led to the isolation of a large number of sesquiterpene lactones having cytotoxic and/or antitumor activity.[1-4] Those compounds which possess *in vivo* antitumor activity are characterized by their polyfunctionality as evidenced by examination of the structures of elephantopin (1),[5] euparotin acetate (2),[6] and vernolepin (3).[7] The importance of the α-methylene-



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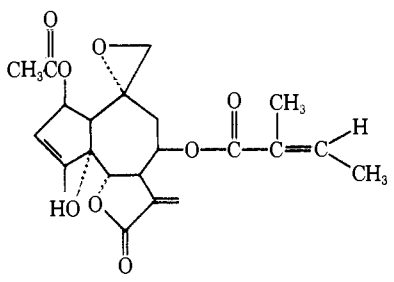

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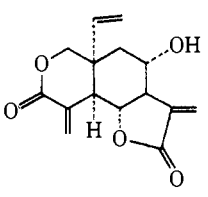

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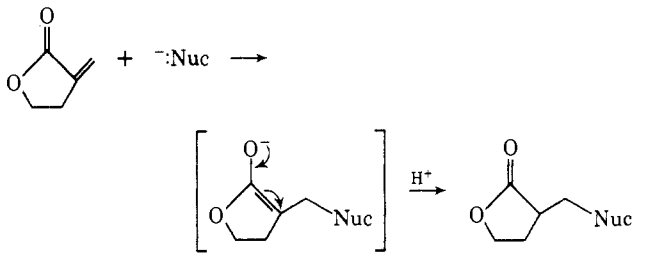
γ-lactone moiety to the activity of these and related compounds has been demonstrated in a series of studies by Kupchan and coworkers.[1] For example, saturation of the conjugated double bond in the α-methylene-γ-lactone groups of vernolepin and elephantopin results in loss of cytotoxic activity. The α-methylene-γ-lactone group appears to act as an alkylating agent by virtue of the Michael addition of biological nucleophiles across the conjugated double bond as depicted in Scheme I.[8]

The usefulness of the natural sesquiterpene lactones has been limited by their relatively high toxicity. In the natural compounds the β-carbon (alkylating site) of the α-methylene lactone system is unsubstituted. A substituent at this position should produce changes in physical properties and in chemical reactivity which could alter biological activity and therefore have important biological implications. In support of this concept Semonsky and coworkers[9] have demonstrated that variation of substituents at the α and β carbons of γ-crotonolactones has significant effects on antitumor activity. We have therefore undertaken the synthesis of a variety of substituted as well as unsubstituted α-methylene-γ-lactones in an attempt to systematically vary the electronic and steric environment at the β position of the conjugated lactone. Because of the apparent relationship between polyfunctionality and *in vivo* antitumor activity, the synthesis of difunctional compounds has been emphasized. The monolactones reported were designed to serve as model compounds and as precursors to mixed difunctional alkylating agents. The utility of mixed difunctionality among certain alkylating agents has recently been demonstrated.[10]

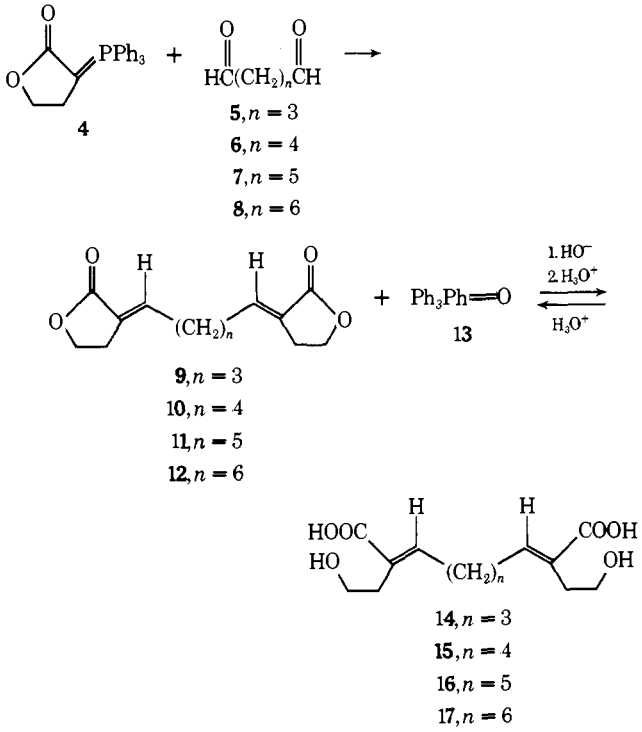
Scheme I



Synthesis. To explore the effect of alkyl substitution on antitumor activity, α,β-unsaturated dilactones 9–12 were synthesized (Scheme II). The range of distances between potential alkylating sites in this series encompasses those found between potential alkylating sites in all of the known natural sesquiterpene lactone tumor inhibitors. The general approach to the preparation of these and related compounds involved the Wittig reaction between ylide 4 and the appropriate aldehyde. The preparation of this ylide[11] and its reaction with aromatic aldehydes to give substituted γ-lactones[12] have been reported. This route appeared to offer easy extension to a variety of alkyl-substituted γ-lactones under relatively mild reaction conditions.

The aldehyde precursors to lactones 9–12 included 1,5-

Scheme II

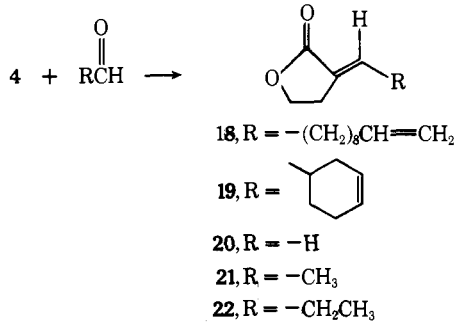


pentanedial (**5**) which was available commercially as an aqueous solution and which was suitable for use upon extraction and distillation. Dialdehydes **6**–**8** were obtained by low-temperature ozonolysis of the appropriate cycloalkenes.

The reaction between **4** and the dialdehydes (Scheme II) occurred readily in benzene at room temperature and was complete when the benzene-insoluble ylide had disappeared to give a clear solution. The most practical method for separation of triphenylphosphine oxide (**13**) from product involved heating the mixture in aqueous base followed by filtration to remove base insoluble **13**. Acidification of the cooled aqueous filtrate resulted in precipitation of the corresponding dihydroxy diacids **15**–**17** which were then reconverted to dilactones **10**–**12** by stirring in dilute aqueous acid at room temperature. Hydroxy acid **14** was sufficiently water soluble that dilactone **9** rather than the hydroxy acid precipitated on standing.

Monofunctional lactones **18**–**19** were prepared (Scheme III) both as reference lactones for comparison of monofunctional *vs.* difunctional activity and as intermediates for later conversion to epoxy lactones. Separation of monofunctional lactones from **13** was effected by distillation, although yields of pure **18** were reduced considerably by eventual codistillation of **13**. The formation of α-methylene-γ-butyrolactone (**20**) by reaction of **4** with formaldehyde represents a previously unreported route to unsubstituted α-methylene lactones. The preparation of compounds of this type has received considerable attention in the recent literature.[13]

Scheme III



No reaction was observed between **4** and 2,5-hexanedione, even after long reflux in toluene. In contrast, reaction of **4** with 3-cyclohexene-1-carboxyaldehyde to give **19** was complete within 2 hr in refluxing toluene.

Assignment of *E* stereochemistry to all products was based on several considerations. House and Rassmusson[14] showed the product from reaction of acetaldehyde and methyl α-(triphenylphosphoranylidene)propionate in methylene chloride to be a mixture of two isomers, 96.5% of which was the isomer having the carbonyl group trans to the alkyl group. By analogy, the *E* isomer would be expected from reactions with ylide **4** in nonpolar solvents. Furthermore, the observed chemical shift at δ 6.7 for the lactone vinyl proton is in close agreement with the value of δ 6.66 predicted on the basis of vinyl substituent deshielding parameters[15] and the observed δ 6.2 absorption of the analogous proton in α-methylene-γ-butyrolactone (**20**).

No *Z* isomer vinyl absorption (*ca.* δ 6.1 predicted) was observed in the nmr of crude reaction products when benzene was used as solvent. However, the appearance of a small δ 6.1 absorption in the nmr of **19** when the reaction solvent was either dimethyl sulfoxide or refluxing toluene was consistent with formation of a small amount of the *Z* isomer under these conditions. House and coworkers reported an increase in *Z* isomer formation from reaction of a stabilized ylide with an aldehyde either in polar protic solvents or in the presence of lithium salts.[16] After stirring for 2 days in methanol and in methanol containing lithium chloride, a mixture of **4** and 3-cyclohexene-1-carboxaldehyde gave predominantly recovered starting material. Similar results were obtained after 5 days in refluxing methanol or refluxing methanol containing lithium chloride. Reaction in dimethylformamide containing lithium chloride was complete in 2 days at room temperature, but the position of the vinyl proton absorption in the nmr of the isolated product was consistent with exclusive formation of the *E* isomer.

Propionaldehyde, an aldehyde less sterically hindered than 3-cyclohexene-1-carboxaldehyde, reacted readily with **4** at room temperature in benzene, methanol, dimethylformamide containing lithium chloride, or dimethyl sulfoxide. Again in these cases examination of the nmr spectra of the reaction mixture suggested exclusive formation of the *E* isomer.

Biological Activity. Monolactones **18**–**20**, dilactones **9**–**12**, and hydroxy acids **15**–**17** were screened according to standard protocols[17] by the National Cancer Institute. They bear NSC No. 166114-166122 and 205367. All of the compounds were inactive against L1210 lymphoid leukemia at doses up to 400 mg/kg. In testing against cells derived from human carcinoma of the nasopharynx (KB), the compounds tested showed ED_{50} values ranging from 16 to 110 μg/ml. Although none of these compounds are considered active by NCI protocols, it is instructive to consider the relative activities in this series. The most active compound is the simple α-methylene-γ-butyrolactone (**20**) which showed an ED_{50} = 16 μg/ml. Thus, although the presence of a single α-methylene-γ-lactone has been shown to be necessary to impart activity (ED_{50} < 4 μg/ml) in a number of sesquiterpenes,[1] it is apparently not sufficient for imparting significant cytotoxicity on the basis of the relative inactivity of **20**. Alkyl substitution at the α-methylene group of the conjugated γ-lactone has a deactivating effect. For example, compounds **18** and **19** show ED_{50} values of 32 and 110 μg/ml, respectively. The difunctional lactones exhibit activity in the range from 40 to 100 μg/ml and so the usual enhancement in activity

Table I

Compound	Reaction time, days	Yield, %	Recrystn solvent[a]	Mp, °C[b]	Formula[c]
(*E,E*)-3,3′-(1,5-Pentanediylidene)bis[dihydro-2(3*H*)-furanone] (**9**)	8	50[d,e]	EtOAc	93.8–95.0	$C_{13}H_{16}O_4$
(*E,E*)-3,3′-(1,6-Hexanediylidene)bis[dihydro-2(3*H*)-furanone] (**10**)	6	70	EtOAc	75.0–76.2	$C_{14}H_{18}O_4$
(*E,E*)-3,3′-(1,7-Heptanediylidene)bis[dihydro-2(3*H*)-furanone] (**11**)	6	80[f]	Et_2O, THF	48.2–49.0	$C_{15}H_{20}O_4$
(*E,E*)-3,3′-(1,8-Octanediylidene)bis[dihydro-2(3*H*)-furanone] (**12**)	13	76	EtOAc	70.0–71.0	$C_{16}H_{22}O_4$
2,9-Bis(2-hydroxyethyl)-*trans,trans*-2,8-decadiene-1,10-dioic acid (**15**)	1	87	EtOH	168.5–170.5	$C_{14}H_{22}O_6$
2,10-Bis(2-hydroxyethyl)-*trans,trans*-2,9-undecadiene-1,11-dioic acid (**16**)	7[g]	62	CH_3CN, EtOH	116.0–117.2	$C_{15}H_{24}O_6$
2,11-Bis(2-hydroxyethyl)-*trans,trans*-2,10-decadiene-1,12-dioic acid (**17**)	3[g]	67[d]	CH_3CN	147–148	$C_{16}H_{26}O_6$
(*E*)-3-(Undec-10-en-1-ylidene)dihydro-2(3*H*)-furanone (**18**)	6[g]	30[h]		139–141 (0.04 mm)[i]	$C_{15}H_{24}O_2$
(*E*)-3-(Cyclo-3-hexenylmethylene)dihydro-2(3*H*)-furanone (**19**)	3	67[h]		110–114 (0.005 mm)[i]	$C_{11}H_{14}O_2$

[a] No solvent mixtures were used; where several solvents are listed recrystallization was from first one and then the other of the indicated solvents. [b] For analytical sample. [c] Infrared and nmr spectra are consistent with the assigned structures; analyzed for C and H. [d] First crop from recrystallization of crude solid obtained in quantitative yield. [e] Based on **4**; hydroxy acid not isolated. [f] Based on unrecovered hydroxy acid. [g] Not optimized; a shorter reaction time is feasible. [h] After two distillations. [i] Boiling point.

seen with unsubstituted dilactones does not occur in the alkyl-substituted series. In order to establish whether monoalkyl substitution can be tolerated to any degree at the site of biological alkylation, the known monolactones **21** and **22** have been prepared and are currently under test. Additional work exploring the utility of alkyl-substituted α-methylene lactones in conjunction with other functional groups (*e.g.*, epoxide) is in progress.

Experimental Section

All melting points were determined on a Mel-Temp apparatus and are uncorrected. The structures of compounds are supported by their ir, nmr, and uv spectra. With the exception of ylide **4** no attempt was made to maximize yield; variations in yields reflect care taken in recovering product from successive mother liquors during recrystallization. Solutions were dried (Na_2SO_4) and concentrated under reduced pressure on a rotary evaporator. Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

α-(Triphenylphosphoranylidene)-γ-butyrolactone (4). α-Bromo-γ-butyrolactone, bp 128–134° (10 mm), was obtained as described by Livak, *et al.*,[18] in 75% yield after two distillations. Reaction with triphenylphosphine was effected on a 1.0-mol scale in 400 ml of THF by the method of Fliszar, *et al.*;[11] by periodically (2, 4, and 30 hr) cooling and filtering the solution, 61–72% yields of crude phosphonium salt were obtained. Dropwise addition of 10% aqueous NaOH to a stirred aqueous suspension of crude salt followed by extraction with excess $CHCl_3$ and crystallization from $CHCl_3$ by successive concentration gave **4** in 80% yield.

Glutaric Dialdehyde (5). A sample of **5** (152 g of a 25% aqueous solution, 0.38 mmol) was saturated with $CaCl_2$ while being cooled in an ice bath. Extraction with EtOAc (4 × 200 ml), drying, filtration, and concentration gave 31.8 g of crude liquid which was distilled to give **5** (12.2 g, 32%), bp 35° (0.15 mm) [lit.[19] 75–80° (18 mm)].

Dialdehydes 6–8. Dialdehydes **6** (86%), **7**, and **8** (43%) were obtained as DMSO solutions by ozonolysis of the appropriate cycloalkene.[20] Direct utilization of the DMSO solutions was possible but necessitated estimation of aldehyde concentration from the nmr spectra. Pure **7** (49%), bp 48–52° (0.07 mm), was obtained by stirring its DMSO solution in dilute acid for 4 days at room temperature followed by EtOAc extraction, washing with aqueous $NaHCO_3$ and with H_2O, drying, filtering, concentrating, and distilling.

Dihydroxy Diacids 15–17. A mixture of freshly distilled dialdehyde (35 mmol), **4** (71 mmol), and C_6H_6 (150 ml) was stirred at room temperature for the time indicated (Table I). Solvent was removed, 10% aqueous NaOH (100 ml) was added, and the mixture was heated 2 hr on a steam bath with vigorous mechanical stirring. After cooling (ice bath), **13** was removed by filtration and the filtrate was washed with $CHCl_3$ (4 × 50 ml). Acidification of the cold solution with concentrated HCl and filtration gave **15–17** as white solids which were recrystallized from the indicated (Table I) solvents.

Dilactones 9–12. A suspension of diacid **15–17** (20 mmol) in 5% aqueous HCl (200 ml) was stirred at room temperature for the indicated (Table I) time. The white solid obtained by filtration was dissolved in $CHCl_3$ (400 ml), washed with 5% aqueous NaOH (2 × 25 ml) and H_2O (5 × 25 ml), dried, filtered, and concentrated to give **10–12** as white solids which were recrystallized from the indicated (Table I) solvents.

Dilactone **9** was obtained as above by stirring at room temperature the acidified solution from which hydroxy acid **14** failed to precipitate.

Lactone 18. A mixture of freshly distilled 10-undecenal (13.5 g, 80 mmol), **4** (27.7 g, 80 mmol), and C_6H_6 (100 ml) was stirred 6 days at room temperature. Concentration and vacuum distillation gave crude **18** (9.3 g, 49%) which was then redistilled (Table I).

Lactone 19. A mixture of 3-cyclohexene-1-carboxaldehyde (5.5 g, 50 mmol), **4** (17.3 g, 50 mmol), and DMSO (75 ml) was stirred 3 days at room temperature. Distilled H_2O was added and the aqueous solution was extracted with $CHCl_3$ (4 × 100 ml). The combined extracts were dried, concentrated, and distilled to give crude **19** (6.9 g, 77%) which was then redistilled (Table I).

Lactone 20. A mixture of aqueous formaldehyde (10 ml of 38% solution, 0.13 mol), **4** (17.3 g, 50 mmol), and Me_2CO (75 ml) was stirred at room temperature for 21 hr after slight initial cooling. The solution was dried, filtered, concentrated, and distilled directly to give pure (by nmr) **20**[21] in 86% yield.

Lactone 19 and 22 for Nmr Study. A mixture of aldehyde (10 mmol), **4** (3.5 g, 10 mmol), and solvent (75 ml) was treated in the indicated (see Synthesis section) manner. DMSO and DMF solutions were poured into H_2O (200 ml) and washed with C_6H_6 (4 × 50 ml), and the combined extracts were washed with H_2O (4 × 25 ml), dried, and concentrated. With C_6H_6, $C_6H_5CH_3$, and CH_3OH as solvent, the reaction mixture was concentrated directly. Trituration with n-C_6H_{14} (2 × 50 ml) and concentration of the hexane solution gave lactones **19** and **22**. These lactones were not further purified but were used directly in obtaining nmr spectra.

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Synthesis and Some Biological Activities of Substance P†

George H. Fisher, John Humphries, Karl Folkers,*

Institute for Biomedical Research, The University of Texas at Austin, Austin, Texas 78712

Bengt Pernow,

Department of Clinical Physiology, Karolinska Institute, Stockholm 60, Sweden

and Cyril Y. Bowers

Tulane University School of Medicine, New Orleans, Louisiana 70112. *Received February 8, 1974*

The undecapeptide, substance P, having the structure H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, has been synthesized by a solid-phase technique on a Beckman automatic peptide synthesizer, appropriately purified, and biologically characterized. In nanogram dosage, synthetic substance P stimulated contraction of the isolated guinea pig ileum, decreased systemic arterial blood pressure, and increased local blood flow in the dog's gracilis muscle. The biological activities of this synthetic undecapeptide are in reasonable agreement with the known activities of preparations of substance P from tissue. Substance P released, *in vitro*, the luteinizing and follicle-stimulating hormones but only at high dosage. Substance P did not release growth hormone, prolactin, or thyrotropin.

In 1931 von Euler and Gaddum[1] described some properties of "an unidentified depressor substance in certain tissue extracts," particularly from intestinal plain muscles and brains of horses. This substance stimulated smooth muscle contraction in some organs and lowered the arterial blood pressure of atropinized rabbits by peripheral vasodilation. A standard preparation of this substance was called "P;" samples also became known as "preparation P" and "powder P." In the following years the expression "substance P" was extensively used.

The active compound, together with other peptides, could be effectively salted out from an aqueous solution by ammonium sulfate[2] and was inactivated by proteolytic enzymes,[3,4] indicating its polypeptide nature. By applying chromatographic separation, Pernow[5] obtained a 1000-fold increase in activity from the starting material of peptides and also showed the presence of substance P in high concentrations in the hypothalamus. It was also found in extracts and subcellular particles of peripheral nerves,[6] pointing to a general localization in nervous tissue.

A number of effects on the central and peripheral nervous system have also been noted.[7,8] Further studies on biological activities and chemical properties of preparations of substance P were reported in the 1960's by various workers.[9,10]

Leeman and Hammerschlag[11] reported in 1967 that extracts of bovine and rat hypothalami contained a peptide that stimulated salivary secretion when such extracts were injected into anesthetized rats. Lembeck and Starke[12] then reported sialogogic activity of their preparations of substance P and suggested that substance P and the sialogogic peptide might be identical.

Chang and Leeman[13] isolated in 1970 the sialogogic peptide from bovine hypothalami and found it to be an undecapeptide with biological properties indistinguishable from those described for substance P. The amino acid sequence was found by Chang, *et al.*,[14] to be H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 and was confirmed by a solid-phase synthesis;[15] the behavior of the synthetic and natural products was identical. In 1973, Studer, *et al.*,[16] isolated substance P from horse intestine and found an amino acid sequence identical with that re-

†Hypothalamic Hormones. 54.